

Application of bio-surfactants as bio-control agents against soil-borne and plant root pathogens

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Biosurfactants are the chemicals that microorganisms produce and are widely used to control plant diseases and industrial level, such as to produce nanoparticles, water purification, molecular imaging etc. Soil-borne pathogens live in the soil for a long time by forming their resting structures such as sclerotia which remain dormant during unfavorable conditions and become active as conditions become favorable. Different fungi form different types of resting structures. Chemical and cultural methods can control these pathogens by applying biocontrol agents and biosurfactants. Biosurfactants are more reliable to control soilborne pathogens because they have low toxicity, biodegradability, control environmental, and specific to their target.

Keywords: biosurfactants, soilborne pathogens, management, biocontrol, application, advantages.

INTRODUCTION

It is important to study plant diseases because they cause damage to plants as well as production. Plant diseases are mainly caused by bacteria, fungi, algae, protozoa, rickettsia-like organisms, nematodes, phanerogams, viruses, and viroid. It is estimated that annual yield losses due to plant diseases are 14% worldwide, with an economic loss of 220 billion \$. The various losses occur between sowing and consumption, in field or storage. The estimated crop losses due to diseases are approximately 30-50% (Pathology, n.d.).

Biosurfactants are surface-active chemicals that are synthesized by microorganisms. Biosurfactants have a wide range of applications. The interest in biosurfactants has raised in recent years because they are specific to the target, easily prepared, and due to their toxicity. Biosurfactants have special functional characteristics that's why they are widely used at the industrial level, such as petroleum, metallurgy, pharmaceuticals, fertilizers, mining, beverages, organic chemicals, agrochemicals, and many other industries. Biosurfactants are produced by yeast, fungi, and bacteria and have low molecular weight compounds. Biosurfactants are also used against mycoplasma activities, insects, viruses, and fungus. In recent years, nanoparticles have been used to produce biosurfactants. The production of these nanoparticles is considered as developing part of green chemistry. Biosurfactants may also use to increase the firmness of microbubble technology. Microbubble technology has a large scope in the diagnosis of disease, water purification,

molecular imaging, and treatment of sewage. The main concern of all countries is to increase the yield of agricultural products to fulfill the food requirements of humans. Food security and sustainable agriculture are carried out by using biological agents as an alternative to synthetic chemicals. In 2004, it is reported that almost ± 0.2 million tons of surfactants are used to protect the crops and for the formulation of agrochemicals (Sachdev and Cameotra, 2013).

The soil-borne pathogen, its importance, and yield loss: It is difficult to estimate the crop loss from pathogens, and the count of losses from soil-borne pathogens is even more tough, because diagnose of soilborne pathogen symptoms is not easy. It is estimated that 50% of crop losses are due soilborne pathogens in the US. The comprehensive study of soilborne pathogens have been done of cereals crops to estimate crop losses.

The management and control of soilborne pathogens is more difficult as compared to above ground pathogens. Soilborne pathogens survive in the rhizosphere which is an infection court. In the rhizosphere, a pathogen creates parasitic relationship with a plant. Both microfauna and microflora are present in the rhizosphere and results of pathogen infection can be influenced by their interaction with the pathogen. Four pathogens attack plants, but two groups, fungi (Oomycetes and true fungi) and nematode, are major in the soil. Few bacterial groups are soil-borne, which may be due to not surviving for long in the soil because of non-spore-forming behavior. The other reason is that bacteria require an injury or

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natural opening to penetrate plants establish for infection. Such as causal agent of tomato wilt (*Ralstonia solanacearum*) and causal agent of crown gall (*Agrobacterium tumefaciens*). Few filamentous bacteria such as streptomycetes are well adapted to live in soil for long period. There are also some viruses which infect the plants through roots. They also require an injury or wound for infection and a vector for the transmission. In soil, viruses are transmitted through zoospore fungi (Olpidium and polymyxa) and nematodes. Morphologically, oomycetes are like fungi, but phylogenetically close to brown algae. The cell wall of oomycetes is composed of cellulose and produce zoospores (swimming spores). Oomycetes are considered as fungi because they have similar mechanism of action as contain true fungi. Almost All fungi which are soilborne, necrotrophic in nature. Most of the biotroph pathogens are, present on above ground parts of plants (powdery mildew and rusts), but few root pathogens are also biotrophs. Like *Phytophthora, sojae* is an example of a semibiotroph, and *Plasmopara halstedii* and *Plasmidiophora brassicae* are biotrophs. Most of necrotrophic pathogens have a wide host range while, biotrophic pathogens have narrow host ranges and these are *Phytophthora, sojae* is an example of a semibiotroph, and *Plasmopara halstedii* and *Plasmidiophora brassicae* are biotrophs gene for resistance.

Among the soil borne diseases, Sheath blight (ShB), caused by *Rhizoctonia solani*, is one of the highly destructive diseases of rice. The pathogen is challenging to manage because of its soil borne nature, extensively broad host range and high genetic variability and due to the inability to find any satisfactory level of natural resistance from the available rice germplasm. The estimated yield reduction from sheath blight ranged from 20% to 42% in artificially inoculated field plots. The use of high doses of nitrogen fertilizer and the introduction of semidwarf high-yielding varieties (HYV) caused a sharp rise in the incidence of sheath blight disease. Higher crop densities and resultant humid canopies have long been advocated as important factors which favor increasing sheath blight incidence. The pathogen is generally soil-waterborne and infectious to a range of plants from ~32 taxonomic families. A typical disease inoculum is sclerotia or runner hyphae from the infected plants. On rare occasions, basidiospores act as inoculum. These sclerotia may remain dormant for over the years in the soil and stubbles and can re-infect healthy rice plants in the subsequent crop season. In addition to the broad host range and variability of the pathogen, the main obstacle in managing sheath blight disease is the lack of an identified germplasm with an adequate level of resistance for using in the resistance breeding programme. Disease control is highly dependent on chemical fungicide, and cultural practices since resistance breeding remain unsuccessful till the date of owing to the inability to identify any resistance resources from the available rice germplasm. Moreover, high genetic variability, extensive host

compatibility and the ability of the pathogen to survive form one crop season to next by forming dormant sclerotia in soil made an additional difficulty in controlling the pathogen. The biological control strategy can be helpful in the control of this soil borne disease. Few previous studies have been reported in which Trichoderma and Streptomycetes spp. were used. However, no specific study for using biosurfactant against sheath blight was reported.

Environmental conditions of soil do not favor the fungus because of extreme dry conditions or due low or high temperature. For survival, the pathogen forms its resistant structure which can be further produce, such as sclerotia, chlamydospores, thick walled hyphae or conidia or survive in crop residues and in plant roots. When roots or seeds approaches dormant structure and conditions become favorable, the fungus is stimulated for germination. First, attachment take place of zoospores or germ tube to the root surface. After which penetration occurs and infects epidermal cells of primary roots, root hairs, and secondary roots or it can attack seedlings and emerging shoots radicles. Seeds can be attacked by some fast-growing pathogens (Pythium species) before emergence. Fungi use mechanical turgor pressure and cell wall degrading enzymes for penetration through intact cell wall. After penetration fungi colonize themselves in root cortex. When the roots are completely killed, the fungus divide through cortex and within the root tissues form spores by reproduction. In this way mycelium of fungus continue its growth internally or externally or it can affect to other close roots. Penetration of wilt disease causing pathogens (e.g. *Verticillium dahlia* *Fusarium oxysporium*) take place into vascular tissues via endodermis and move up into xylem. This way, flow of water stops at other above ground parts of the plant. It is difficult to diagnose the symptoms of soilborne pathogens, because their symptoms above the ground are similar to those symptoms which are caused by abiotic factors (e.g. lack of nutrients drought and stress) and mostly their symptoms are present on roots or below ground (Raaijmakers & Paulitz, 2009).

MANAGEMENT STRATEGIES FOR SOIL-BORNE PATHOGEN

The management strategies of soilborne pathogens depends upon information and knowledge about host, pathogen and environmental conditions that favor the development of disease. These three factors must be present for the development of disease. To infect a susceptible host, a virulent pathogen must have enough inoculum. e.g., zoospores. And for the growth of pathogen and plant, the environmental conditions must be favorable for infection. Saturated soil for certain periods favors the development of water molds and infects the roots. The applications of control measures at the time is also critical factor. Such as, phytophthora root rot can be controlled through early implementation of control measures.

Resistant cultivars: Resistance to many pathogens long lasting and complete. That's why, it is important control measure. Immunity is produced in the host plant through expression of single genes. It is called vertical resistance. Vertical resistance controls the production of inoculum and initial level of infection. Such as resistance against certain races of fusarium wilt. The resistance is produced in plants by incorporating multiple genes called horizontal resistance. It is a broad resistance against many pathogens. In case of horizontal resistance, some disease can develop but to a tolerable level. Use of resistant varieties is best strategy if they are available.

Cultural practices: Such conditions that are unfavorable for disease development should be created. Reduced the inoculum where the host plant is present. Those cultural practices should be adopted which reduce host-pathogen contact, such as using fields with no history of soil-borne pathogens, disease-free transplants or seeds should be used and prevent the pathogen spread from the infected to the healthy field. Transplants and seeds should be planted to a proper depth.

Soil solarization, proper irrigation, crop rotation and good sanitation are the examples of cultural practices that reduce the inoculum in environment. Some brassica crops and cover crops reduce the populations of soilborne pathogens. When these crops are incorporated into the soil, their residues enhance soil microflora that compete with soil pathogens and release chemicals that inhibits pathogens. Unfavorable can be created by good soil drainage, reducing soil humidity through optimum plant spacing, irrigation practices, using proper fertilizer to prevent stressed and by using mulches. Drip irrigation is a good example of cultural practice used in vegetable crops. It allows precise delivery of water which results in better water management. Drip irrigation reduced soil saturation and reduced the risk of soil-borne pathogens. For example, root rots.

Chemical control: Agricultural chemicals are also included in soil-borne pathogens' management strategy. Pre-plant fumigants are highly successful in reducing the inoculum of soilborne pathogens. But pre-plants fumigants are strictly regulated and expensive in use. Seed and seedling diseases can be controlled by treating the seeds with fungicides. In some cases, fungicides are applied to the plants or soil for disease management. Such as, the application of fungicides can control damping-off disease caused by *Pythium* when planting to spinach seed lines. However, Application of fungicides into the field is not very effective against most soilborne pathogens (Koike et al., 2016). The soilborne fungal pathogens can be controlled effectively through benzimidazole, dicarboximide and triazole fungicides groups. *Rhizoctonia solani* is often controlled using azoxystrobin fungicides. Different modes of action should be adopted in the rotation of the fungicides program to minimize the risk of resistant development. Using fumigants and fungicides is the

last step in controlling soil-borne pathogens. Health of the crops, aesthetics and marketability are adversely affected by the use of fungicides and fumigants (Panth et al., 2020).

Biological control: It is also under the process of development. In this strategy, a microorganism containing antagonistic effect is applied to compete with the target pathogen. Biological control is not effective for most soilborne pathogens and not yet available (Koike et al., 2016). The biocontrol agents damage soilborne pathogens through production of antagonistic chemicals, induction of resistance against pathogens, parasitism and competition for nutrients and host. *Pseudomonas*, *Paecilomyces*, *Gliocladium*, *Rhizobium*, *Bacillus*, *Trichoderma*, *Serratia* and *Streptomyces* are the spp. of bacteria and fungi which are used as a biological control against soilborne pathogens. Soilborne pathogen which caused root rot is effectively controlled by using *Trichoderma viride*, *Fluorescent*, *Pseudomonas*, *Bacillus subtilis* and *T. harzianum* in many crops. Fungi-toxic metabolites are produced in large quantity by the *Trichoderma* species. These metabolites are active mycoparasites which are effectively used as a biological control against plant parasitic soilborne nematodes, soilborne and foliar diseases. Arbuscular mycorrhizal fungi are also used as a biological control against soilborne diseases. Although, biocontrol agents are used in sustainable agriculture and its efficacy highly dependent on integrated approaches (Panth et al., 2020).

Efficacy As Biocontrol Agent: Infectious structures of oomycetes pathogens are zoospores, and these structures may be devastating, especially in the case of the hydroponic system. Miller and Stanghellini found the biosurfactants as a biological agent in 1997, who proved that the membrane of zoospores could be interrupted by rhamnolipids and as a result, lysis takes place in zoospores of many oomycetes pathogens of plants. The study of several reports showed that zoospores producing pathogens could be suppressed by using rhamnolipids. The cyclic lipopeptides (CLPs) contain antibiotic effects, and many harmful effects have been demonstrated on different plant pathogens using CLPs. In the pesticides market, several strains of *Bacillus* are used in biocontrol. There are many cases in which we can't understand whether disease suppression is due to CLPs production. The role of CLPs of *Bacillus* has been summarized as a biocontrol in many studies. *Pseudomonas* is not used commercially as *Bacillus* in biocontrol because *Bacillus* is easier to handle and their spore-forming characters. *Pseudomonas* produces Biosurfactants with strong antibiotic effects in controlled conditions (invitro).

Application Of Biosurfactants For The Elimination Of Pathogens: Biosurfactants are considered as biocontrol agent for the achievement of sustainable agriculture. Because many plant pathogens can be controlled by the biosurfactants that are obtained from microbes and have antimicrobial activity.

The application of biosurfactants control pathogens through antibiosis, induced systemic resistance, parasitism and hypovirulence. Mostly, the surfactants are used to increase antagonistic activities of microbial products and microbes. There are some surfactants which inhibit the production of aflatoxin from *aspergillus* spp, infecting peanuts, corn, and cotton in the field and during storage. Thus, biosurfactants directly or indirectly play an important role in eliminating plant pathogen.

Dickeya spp. and are effectively controlled by the biosurfactant which are produced by the rhizospheric isolates of *Bacillus* and *Pseudomonas*. The plant pathogens which form zoospores have acquired resistance to synthetic chemicals. The rhamnolipids effectively inhibit these zoospores forming plant pathogens. Another study shows that rhamnolipids can stimulate plant immunity, reducing the infection by plant pathogen. The biosurfactants produced by the plant growth promoting *Pseudomonas* cause lysis of zoospores *Phytophthora capsica* which belongs to oomycete and causative agent of cucumber. The *Bacillus* strains produces lipopeptide which inhibit the growth of plant pathogenic fungi such as *Biopolaris sorokiniana* *Fusarium* spp. and *Aspergillus* spp. The strains of *Pseudomonas fluorescens* produce biosurfactants that have antifungal properties.

In in vitro conditions, the growth of *verticillium* spp. is inhibited by the biosurfactants produced by *Pseudomonas* spp. The growth of *Phythium ultimum* and *Rhizoctonia solani* is inhibited by the production of dual functioning compounds viscosinamid, tensin and viscosin from *Pseudomonas* sp. *Colletotrichum gloeosporioides* cause anthracnose on papaya and it is controlled by the biosurfactants which are produced by the *Bacillus subtilis*. The dual function involves antifungal activity and biosurfactants.

It has been reported that biosurfactants produced by the *Staphylococcus* sp. inhibit the plant pathogen *Pseudomonas aeruginosa*. Hence, these biosurfactants are the best alternative of harsh chemical pesticides which are being currently used in agriculture. Biosurfactants are also enhance the composting process because they provide favorable conditions for microbial growth. It is an additional advantage of these biosurfactants (Sachdev and Cameotra, 2013).

Advantages of biosurfactants: There are the following advantages of biosurfactants when compared with synthetic surfactants.

Biodegradability: Biosurfactants are easily biodegradable. Biodegradable is the ability of a product to break down into small basic components through natural processes of fungi, bacterial and other microorganisms. Due to concerns about environmental pollution, biodegradation is an important issue. Biodegradation is favorable for the environment because it does not cause environmental pollution.

Low toxicity: Biosurfactants are not harmful for living organisms and easily tolerated, because their toxic level is

low. While on the other hand chemical surfactants/synthetic surfactants are harmful for living organisms due their high toxicity. Because of the non-toxic or less toxic effects on living and no effect on bioactivity of living organisms, they are appropriate for use in cosmetics, food and pharmaceutical.

Accessibility to raw material: Cheap raw material can be used to produce biosurfactants, such as potato process effluents, cassava flour wastewater, oil of sunflower, oil of rapeseed and waste of oil refinery etc. These raw materials are easily available in huge quantity. The carbon is obtained from carbohydrates, lipids, and/ or hydrocarbons. And these can be used in the combine form or separately.

Economically acceptable production: Depending upon the use of biosurfactants, these can be produced by products and waste of industries. Biosurfactants are produced in a quantity which is economically acceptable.

Control environmental pollution: Biosurfactants are used to control oil pills, bioremediation of soil, which is contaminated, to handle the emulsions of industries, detoxification and biodegradation of industrial wastes.

Specificity: Biosurfactants are complex organic molecules which are specific to their target because, that contain specific functional groups and these functional groups have specific action. It would depend upon interests such as specificity for food, to detoxify specific pollutants and specific for cosmetics etc (Chapter- 1 Introduction, 1999).

Disadvantages of biosurfactants: Cheap and large-scale production of biosurfactants are major problem. Usually, biosurfactants are required in large quantity in environment and petroleum applications. That's why may be expensive due to bulk use. The availability of pure substances is another problem, which is important during food, cosmetic and pharmaceutical applications. There are multiple consecutive steps during the downstream processing. That's why, for the purification and facilitated recovery, biosurfactants concentration and high yields are essential in bioreactors (Engineering, 1992).

Conclusions: Biosurfactants are derived from micro-organisms such as bacteria, fungi, viruses and viroid etc. Biosurfactants have wide range of applications such as in nanoparticles, as biocontrol agents to control soilborne and root borne plant pathogens, pharmaceuticals, fertilizers etc. The reason of their wide applications is their low toxicity, biodegradability, raw products are easily available and economically cheap, environmentally friendly and specific to target. The large volume is required of biopesticides for their application and purification are major drawbacks of biopesticides production.

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